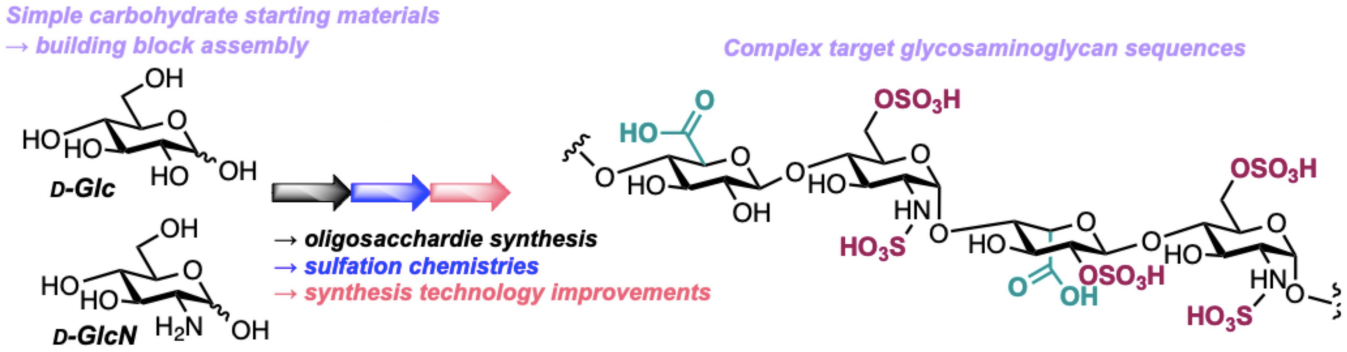
Record Review

THE CHEMICAL RECORD

doi.org/10.1002/tcr.202100173

Developments in the Chemical Synthesis of Heparin and Heparan Sulfate

Imlirenla Pongener, Conor O'Shea, Hannah Wootton, Michael Watkinson, and Gavin J. Miller*[a]



Abstract: Heparin and heparan sulfate represent key members of the glycosaminoglycan family of carbohydrates and underpin considerable repertoires of biological importance. As such, their efficiency of synthesis represents a key requirement, to further understand and exploit the H/HS structure-to-biological function axis. In this review we focus on chemical approaches to and methodology improvements for the synthesis of these essential sugars (from 2015 onwards). We first consider advances in accessing the heparin-derived pentasaccharide anticoagulant fondaparinux. This is followed by heparan sulfate targets, including key building block synthesis, oligosaccharide construction and chemical sulfation techniques. We end with a consideration of technological improvements to traditional, solution-phase synthesis approaches that are increasingly being utilised.

Keywords: carbohydrates, heparan sulfate, heparin, glycosaminoglycan, glycosylation

1. Introduction

Carbohydrates are indispensable to glycoconjugate biological function; this is typified by the glycosaminoglycans (GAGs). GAGs are present on most animal cell surfaces and in the surrounding extracellular matrix. They are extremely diverse, containing a linear and structurally heterogeneous anionic glycan chain and impart important biological functions by binding to different growth factors, enzymes, morphogens, cell adhesion molecules, and cytokines. One GAG in particular, heparan sulfate (HS) is involved in mediating mammalian cell function, exemplified by its interaction with growth factors such as fibroblast growth factors (FGFs), a protein family involved in cell proliferation, differentiation, and angiogenesis.[1] HS also mediates many pathological conditions including cancer,[2] Alzheimer's disease,[3] viral infections such as SARS-Cov-2,[4,5] HIV[6] and HSV,[7] alongside numerous bacterial infectivity events.[8] Structurally related to HS is the anticoagulant heparin (H), present within mast cells and considered a highly sulfated, tissue-specific variant of HS.

The chemical structures of H and HS (Figure 1) consist of repeating disaccharide units, composed of glucosamine (D-GlcN) and a uronic acid (D-GlcA). Its microstructure is diverse: the amino sugar can be *N*-sulfated (D-GlcNS) or *N*-acetylated (D-GlcNAc), D-GlcA can be epimerised to L-IdoA and saccharide units are variably substituted with *O*-sulfate groups, at the C6 (and occasionally C3) of D-GlcN and at C2 of D-GlcA/L-IdoA. HS chains have an average molecular weight of 30 kDa and this, taken in context with the possibilities for functional group variation, presents a huge structural microheterogeneity and enormous scientific challenge in unravelling and understanding the HS structure-to-function paradigm.

Due to their structural complexity and biological importance, the synthetic challenge surrounding access to (and application of) structurally defined H and HS fragments is significant and of continued interest. In this review we focus on chemical approaches to and methodology improvements for the synthesis of H/HS from 2015 onwards. Contextually, this should be considered alongside the impressive and complimentary advances in chemoenzymatic approaches to access H/HS that have been well reviewed elsewhere,[9,10] alongside GAG synthesis approaches in general.[11] We first consider advances towards the synthesis of the heparin pentasaccharide fondaparinux (and analogues), followed by HS targets, including building block synthesis, oligosaccharide construction and chemical sulfation methods. We end with a consideration of technological improvements to traditional, solution-phase synthesis approaches that are increasingly being utilised.

2. Developments in the Chemical Synthesis of Heparin-Related Pentasaccharide Sequences

Synthesis of the pentasaccharide anticoagulant drug fondaparinux **1** (Arixtra) by Sanofi and Organon in 2001 represented a

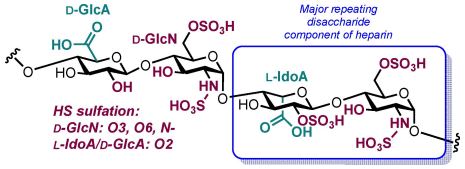


Figure 1. Overview of HS structure, heteroatoms commonly sulfated are shown in magenta (D-GlcN- and -O6 shown), C5 epimeric uronates green. Included within the blue box is the major repeating disaccharide unit of structurally related H. Free acid form shown.

[a] *Dr. I. Pongener, C. O'Shea, H. Wootton, Prof. M. Watkinson, Prof. G. J. Miller*
Lennard-Jones Laboratories, School of Chemical and Physical Sciences, Keele University, ST5 5BG, Staffordshire, UK
E-mail: g.j.miller@keele.ac.uk

landmark achievement in the construction of mimetic heparin oligosaccharide sequences.[12] However, the original 50-step route delivered the target molecule in an overall yield of only 0.1%, presenting an opportunity and requirement for efficiency improvements. We first discuss recent efforts to develop more efficient synthetic routes to **1** using traditional modular synthetic routes and then explore one-pot, programmable methodologies.[13]

2.1. Improving Large-Scale Synthesis of 1

In 2017, Ding and co-workers' reported a practical and efficient large-scale synthesis of **1** using a combination of the Alchemia and Sanofi protocols (Scheme 1).[14] The group identified that the original Alchemia protocol involved simple and practical access to the monosaccharide building blocks **2**–**3** and, therefrom, trisaccharide donor **6** on a large scale. However, a drawback in the glycosidation of **6** with

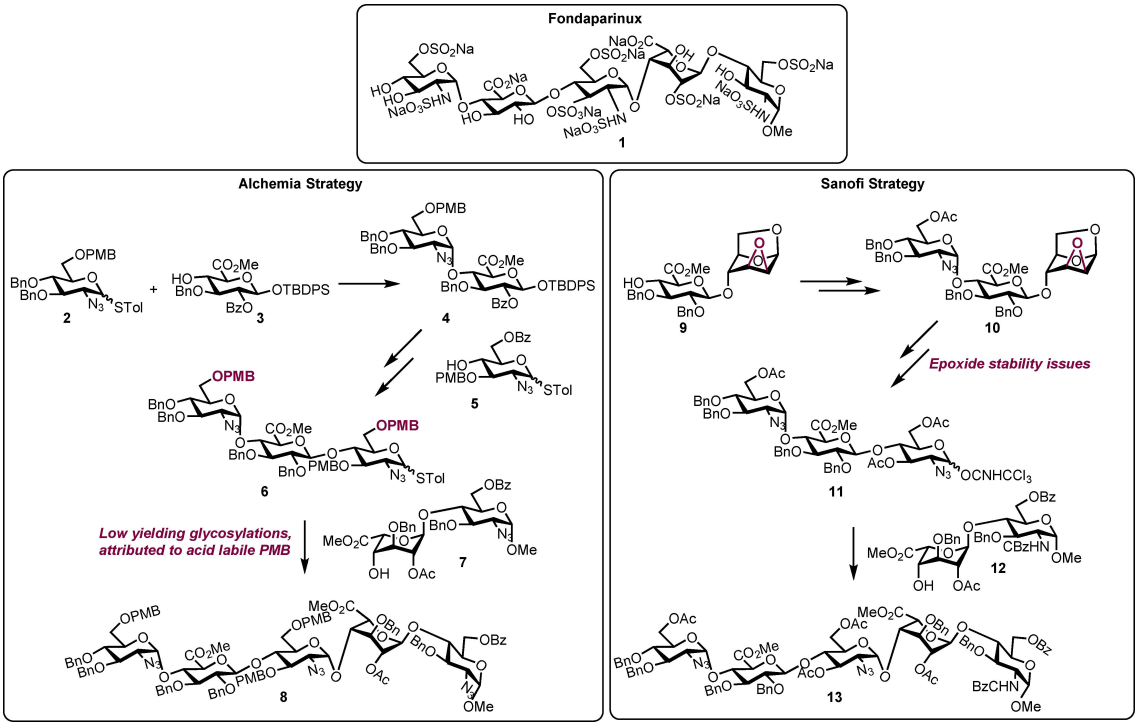
Ren is from Nagaland, India. She completed her BSc at Christ University Bangalore, India. In 2014, she moved to Dublin, Ireland to undertake an MSc in Chemistry from University College Dublin, during which time she carried out research in organocatalysis in the McGarrigle group. She then pursued her PhD in the same group, focusing on methodology development for stereoselective glycosylations. After completing her PhD in 2019, she continued as a PDRA in the McGarrigle group working on beta-bannosylations. She took up her current post as PDRA in the Miller group in 2020, where her research focuses on the chemical synthesis of glycosaminoglycans.

Hannah Wootton was awarded her MChem degree in 2019, with first class honours, from Keele University. Following a year in the pharmaceutical industry, she returned to Keele University in September 2020 to undertake her PhD under the supervision of Prof. Gavin Miller and Prof. Michael Watkinson. Her research primarily involves the synthesis of glycosaminoglycan targets, with subsequent conjugation to fluorogenic components.

Conor graduated from University College Dublin with a B.A. of Science majoring in Medicinal Chemistry and Chemical Biology. His final year research project, under the supervision of Dr. Eoghan McGarrigle, involved the synthesis and design of organocatalysts to achieve stereoselective control in glycosylation reactions. In 2020, he joined the Miller group as a PhD student where his research is focused on the chemical synthesis of glycosaminoglycans.

Mike Watkinson graduated from the University of St. Andrews in 1991 before completing his PhD at UMIST in 1994. After a year as a Royal Society Postdoctoral Fellow at the University of Santiago de Compostela, Spain in 1995 he returned to UMIST before his appointment at Queen Mary, University of London in 1998 where he remained until joining Keele University as the Head of School of Chemical and Physical Sciences in 2018. His current research focuses on the development of small molecule fluorescent probes for a range of sensing applications.

Gavin graduated from UMIST with an MChem in 2001. Continuing his studies at the University of Manchester, he undertook a PhD in synthetic carbohydrate chemistry, followed by a PDRA at St Andrews. Gavin then worked in industry, firstly at Ferring Pharmaceuticals and secondly at Peakdale Molecular before returning to academia and the University of Manchester in 2010. This final PDRA in the Manchester Institute of Biotechnology developed chemical synthesis approaches to heparan sulfate oligosaccharides. He took up his current appointment in organic chemistry at Keele in 2016, where his group focuses on the chemical and enzymatic synthesis of carbohydrates.



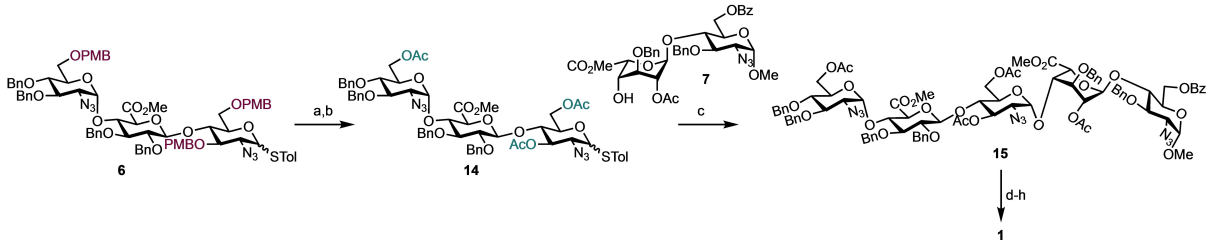
Scheme 1. Overview and issues surrounding Alchemia (left-hand box) and Sanofi (right-hand box) strategies towards the synthesis of **1**.

disaccharide acceptor **7** (to give pentasaccharide **8**) was delivery of the desired material in very poor yield. This was suggested to arise from the use of acid labile PMB protecting groups within **6**. Comparatively, in the case of the Sanofi protocol, glycosylation of acceptor **12** with trisaccharide **11** was successful and observed with excellent diastereoselectivity in delivering pentasaccharide **13**. However, the use Cerny epoxide precursors **9** or **10** were not amenable to large scale application, due to stability issues.

Based on these observations, the group decided to adopt the Alchemia method to access trisaccharide donor **6** for subsequent glycosylation using the Sanofi method (Scheme 2). The acid labile PMB groups within trisaccharide donor **6** were replaced with acetyl groups to provide trisaccharide **14**. Retaining the use of thioglycoside donors, trisaccharide **14** was successfully coupled with disaccharide acceptor **7** to deliver pentasaccharide **15** in 74% yield and on >100 g scale. Pentasaccharide **15** was converted through to **1** in 5 steps in 62% overall yield and on an impressive >30 g scale.

2.2. Iterative One-Pot Syntheses of 1

In 2018, Wong and co-workers' reported a highly efficient and programmable one-pot method for the synthesis of protected



Scheme 2. Ding and co-workers' strategy to synthesise **1** combining aspects of the Alchemia and Sanofi methods. Reagents and conditions: (a) (i) DDQ, CH_2Cl_2, H_2O (10:1), 0–20 °C, 83%, or (ii) CAN CH_3CN, H_2O (20:1), 0 °C, 76%, or (iii) CF_3CO_2H, CH_2Cl_2 (1:10), 0 °C, 73%; (b) (i) Ac_2O, pyridine, 20 °C, 2 h, 92%, or (ii) Ac_2O, Na_2CO_3, 20 °C, 10 h, 84%; (c) NIS, CF_3SO_2OH, CH_2Cl_2, 4 Å MS, −20 °C, 3 h, 74%; (d) NaOH, THF, 0 °C, 24 h, 97%; (e) SO_3·pyridine, pyridine, Et_3N (5:1), 20 °C, 83%; (f) PMe_3, NaOH, THF; (g) SO_3·pyridine, pyridine, Et_3N (5:1), 20 °C, 93%, 2 steps; (h) H_2, Pd/C, MeOH, H_2O (1:1), 83%.

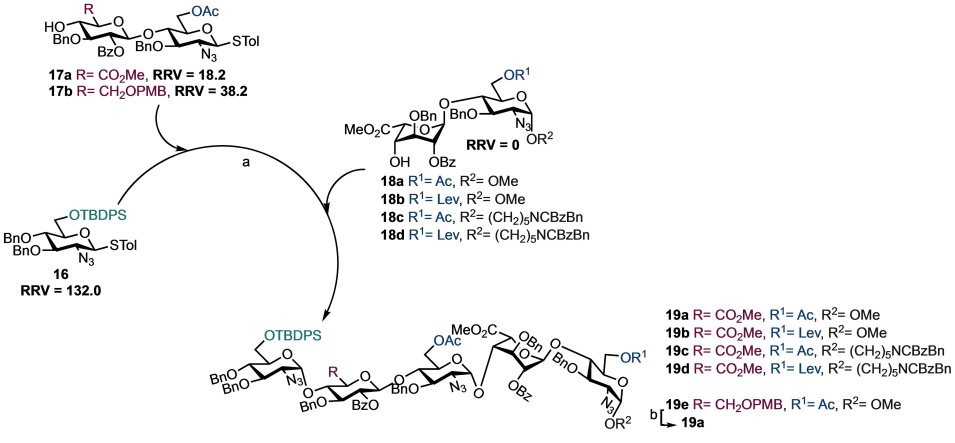
heparin pentasaccharides utilising thioglycoside building blocks (Scheme 3).[15] The group designed eighteen key intermediates with improved relative reaction values (RRVs) for a one-pot synthesis to then access 6-*O* sulfation pattern variant pentasaccharides.

The building blocks were designed to incorporate the following (a) highly stereoselective α-glycosylation outcomes, influenced by ring protecting groups (N_3, TBDPS, and Ac) (b) use of TBDPS as a protecting group at the D-GlcN-*O*-6 position to increase the RRV of donor **16**[16] (c) stereoselective construction of β-1,4-glycosidic linkages using a neighbouring C2-*O*-benzoyl ester and (d) selective installation of a 6-*O*-sulfate using orthogonal acetyl and levulinic protecting groups. To investigate if it was better to introduce the glucuronic acid as part of the disaccharide building block or through oxidation at the pentasaccharide level, **17b** (RRV = 38.2) was chosen as the thioglycoside donor to be used in the one-pot synthesis of pentasaccharide **19e**. This material was subsequently deprotected, oxidised and esterified to generate pentasaccharide **19a** in 42% yield. In contrast, when glucuronic acid containing disaccharide acceptor **17a** (RRV = 18.2) was used, pentasaccharide **19a** was furnished in 54% yield (Scheme 3). Thus, it was concluded here that the pre-glycosylation oxidation was more efficient than a post-glycosylation oxidation (54% *versus* 42% yields respectively).

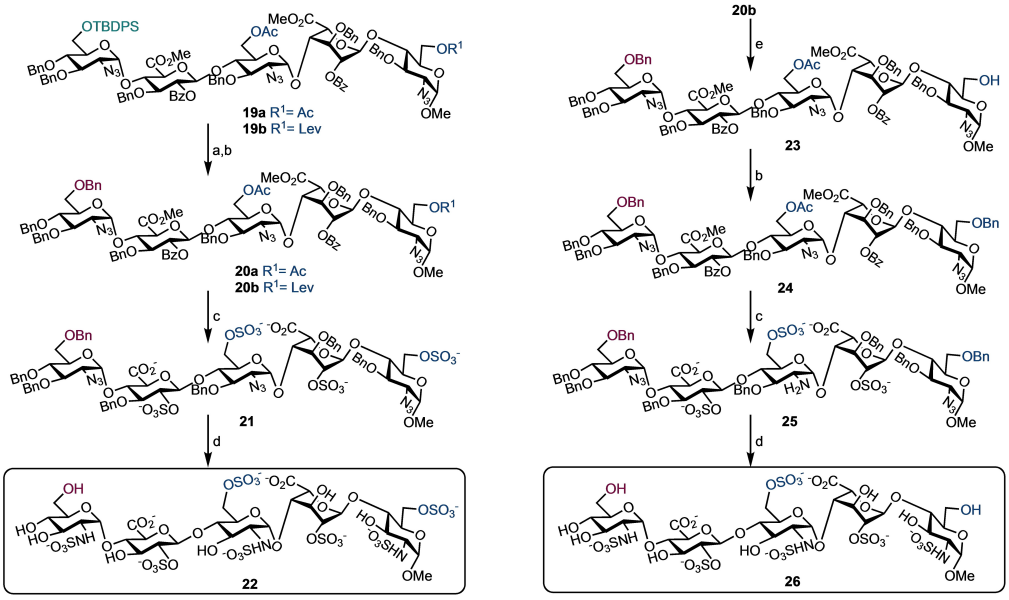
Following the successful synthesis of pentasaccharides **19a–d**, **19a** and **19b** were further used to obtain regiodefined sulfate derivatives **22** and **26** (Scheme 4). Notably, when introducing *O*-sulfates, the group found it crucial to first replace the D-GlcN-*O*-6-TBDPS group in **19a/b** with a benzyl group (**20a/b**), to prevent an unwanted insertion of SO_3 into the Si–O bond. It was also noted that co-solvent-promoted *O*-benzylation using Ag_2O/BnBr in ^{n}Hex : CH_2Cl_2 was found to be an ideal method to avoid acyl migration, ester hydrolysis and sugar decomposition.[17]

Recently, Zhao and co-workers' also developed an optimised route towards **1**, harnessing a preactivation-based, one-pot glycosylation, concomitantly installing two new stereocentres with exclusive α-stereoselectivity (Scheme 5).[18] To synthesise pentasaccharide precursor **30**, monosaccharide thioglycoside donor **27** and disaccharide acceptors **28** and **29** were glycosylated in one-pot using *p*-TolSCl and AgOTf as promotors, delivering **30** in 37% yield. The target compound **1** was obtained following saponification, *O*-sulfation, hydrogenolysis and *N*-sulfation. The total number of synthetic steps was 34, from commercial materials, and the overall yield was 27%; notably demonstrating a simplified route to **1** with improved synthetic efficiency.

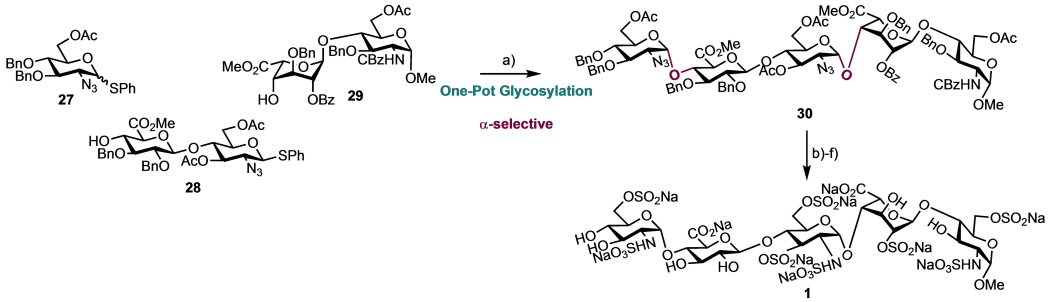
Most recently, Wong and co-workers' reported a potentially scalable and programmable one-pot synthesis of **1** using a [1,2,2] strategy (Scheme 6).[19] This synthesis built on an earlier approach by the group accessing the related anticoagulant drug idraparinux.[20] The building blocks were designed with TBDPS and acetyl at the 6-*O* positions to support α-selective glycosylation. Initial glycosylations using disaccharide acceptor **32a** (R=Bz, Scheme 6) gave a poor yield of 26%, however upon switching to acceptor **32b** (R=Ac, Scheme 6), an improved yield of 50% was achieved. The total synthesis of **1** was achieved in 4.2% yield over a total of 22 linear steps.



Scheme 3. Wong and co-workers', programmable one-pot synthesis of pentasaccharides **19a–d**. Reagents and conditions: (a) NIS, TfOH, CH_2Cl_2, −45 to −25 °C, **19a**: 54%, **19b**: 48%, **19c**: 50%, **19d**: 42%; (b) (i) DDQ, CH_2Cl_2, H_2O (10 : 1), rt, 1 h; (ii) BAIB, TEMPO, CH_2Cl_2, H_2O (2 : 1), rt, 2 h; (iii) MeI, $KHCO_3$, DMF, 0 °C to rt, 4 h, 42%, 3 steps.



Scheme 4. Wong and co-workers', diversification of pentasaccharides to access regiodefined, sulfated derivatives. Reagents and conditions: (a) HF·pyridine, pyridine, 0 °C to rt, 12 h; (b) BnBr, Ag_2O, ″Hex, CH_2Cl_2 (4 : 1), 4 Å MS, 70 °C, 12 h, **20a**: 68 % 2 steps, **20b**: 66 % 2 steps, **24**: 81 %; (c) (i) LiOH, H_2O_2, THF, −5 °C to rt, 8 h; (ii) NaOH, MeOH, rt, 18 h; (iii) SO_3·Et_3N, DMF, 55 °C, 12 h; (d) (i) H_2, $Pd(OH)_2$/C, MeOH, 36 h, rt; (ii) SO_3·pyridine, NaOH, pH 9.5, H_2O, rt, 38 h; **22**: 40 % 5 steps, **26**: 47 % 5 steps; (e) NH_2NH_2·AcOH, THF, MeOH (1 : 1), 0 °C to rt, 2 h, **23**: 85 %.



Scheme 5. Zhao and co-workers' preactivation-based one-pot synthesis of **1**. Reagents and conditions: (a) *p*-TolSCl, AgOTf, −78 to −20 °C, 1 h per glycosylation, 37 %; (b) LiOH, H_2O_2, THF; (c) NaOH, MeOH, 81 %, 2 steps; (d) SO_3·Et_3N, DMF, 65 °C, 24 h, 94 %; (e) H_2, Pd/C, MeOH, H_2O; (f) SO_3·pyridine, NaOH, H_2O, rt, 97 %.

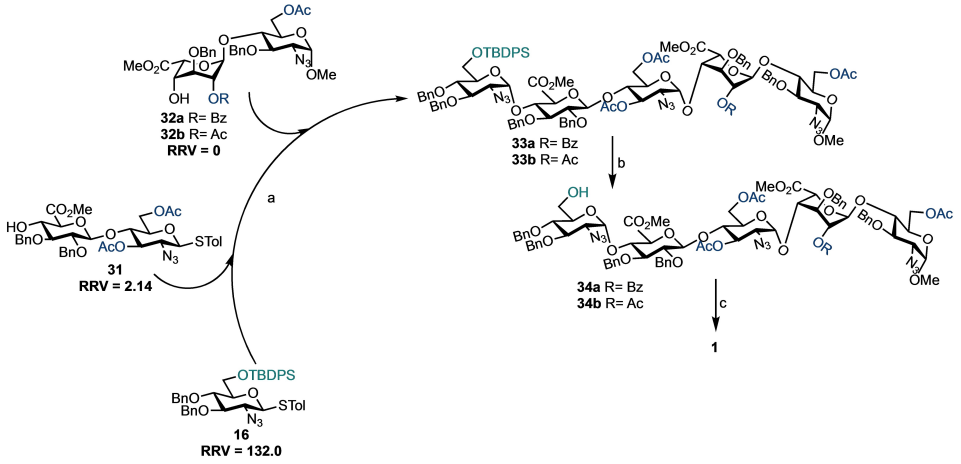
3. Synthetic Methodology Developments for Heparan Sulfate Synthesis

At a basic level, HS consists of a repeating disaccharide unit of either (-L-IdoA-α-1,4-D-GlcN-α-1,4-) or (-D-GlcA-β-1,4-D-GlcN-α-1,4-). Consequently, efforts in HS synthesis usually focus first on the provision of appropriate disaccharide building blocks,[21] for subsequent iterative oligosaccharide assembly. Additionally, *de novo* syntheses of mimetic fragments containing just one monosaccharide component (e.g., L-IdoA) have been completed.[22] With appropriately protected oligosaccharide sequences in hand, final functionalisations are made to incorporate essential *O*- and *N*-sulfate groups. Accordingly, we have divided recent accomplishments adopting this approach into three categories: i) synthesis of HS building blocks ii) oligosaccharide synthesis and iii) concepts to effect *O*- and *N*-sulfation.

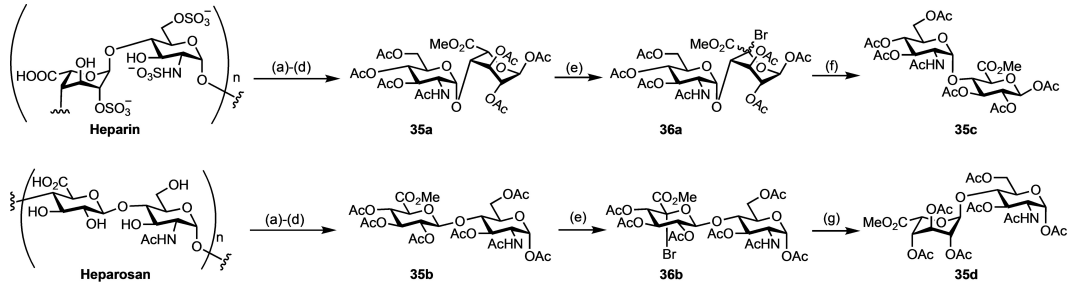
3.1. Synthesis of HS Building Blocks

3.1.1. HS Building Blocks from Polysaccharide Digestion

Hsieh-Wilson and co-workers' have recently described novel access to key HS disaccharide building blocks **35a–d** (Scheme 7).[23] Notably, two of the four core disaccharides generally required for HS assembly were obtained *via* digestion



Scheme 6. Wong and co-workers' programmable one-pot synthesis of **1**. Reagents and conditions: (a) NIS, TfOH, CH_2Cl_2, −45 to −25 °C, 3 h, **33a**: 26%, **33b**: 50%; (b) HF·pyridine, pyridine, 0 °C to rt, 16 h, **34a**: 89%, **34b**: 91%; (c) (i) LiOH, H_2O_2, THF, −5 °C to rt, 16 h then NaOH, MeOH, 0 °C to rt, 18 h; (ii) SO_3·Et_3N, DMF, 55 °C, 16 h; (iii) H_2, $Pd(OH)_2$, MeOH, pH 7, 36 h; (iv) SO_3·pyridine, NaOH, H_2O, 48 h, 67%, 4 steps. For acceptors **32a–b**, no anomeric leaving group is present hence RRVs were not measured and assigned as zero.



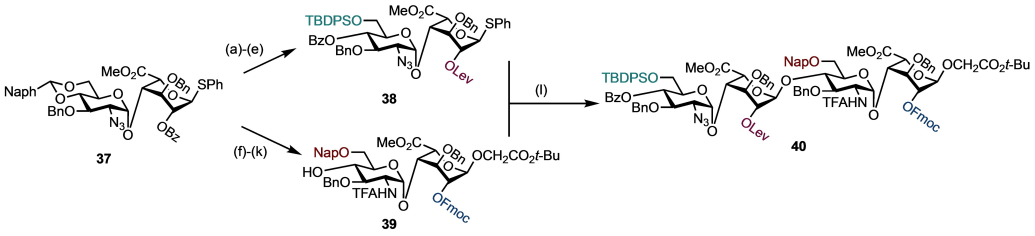
Scheme 7. Hsieh-Wilson's synthesis of four core heparan sulfate disaccharides. Reagents and conditions (a) 1 or 2 M TfOH, H_2O, 100 °C; (b) AcCl, MeOH, 65 °C; (c) Ac_2O; (d) Ac_2O, pyridine, **35a**: 18%, 4 steps, **35b**: 16%, 4 steps; (e) NBS, CCl_4, hv, reflux, **36a**: 80%, **36b**: 75%; (f) Bu_3SnH, AIBN, toluene, 110 °C, 33%; (g) Bu_3SnH, Et_3B, toluene, 20 °C, 63%.

of readily available natural polysaccharides, enabling a cost-effective and scalable entry to such materials. Firstly, heparin was hydrolysed to a crude disaccharide using 2 M TfOH at 100 °C, followed by carboxylate esterification and O/*N*-acetylation in one pot to afford the core D-GlcN-L-IdoA disaccharide **35a** in 18% yield over 4 steps. Importantly, the method alleviated any requirement for effective 1,2-*cis* glycosylation in generating a D-GlcN-α-1,4-L-IdoA building block. Secondly, heparosan was selectively digested at the D-GlcN reducing position within the polysaccharide, thus accessing a related D-GlcA-D-GlcN disaccharide **35b** in 16% yield over four steps and with reaction conditions identical to those used for **35a**, save for a reduced molarity of TfOH. Two further core HS disaccharides, **35c** (D-GlcN-D-GlcA) and **35d** (L-IdoA-D-GlcN), could also be obtained through uronate C5 epimerisation of **35a** and **35b** respectively (Scheme 7).

The authors were also able to access more complex, orthogonally protected disaccharide building blocks, exemplified by **37**, in just nine steps from heparin (Scheme 8). Disaccharide **37** then underwent strategic protecting group manipulations to afford both donor **38** and acceptor **39** components. These were then combined to access tetrasaccharide **40** in a 60% yield (Scheme 8). The incorporation of seven different protecting groups into **40** uniquely allowed each L-IdoA-2-*O*, D-GlcN-6-*O* and *N*- to be unmasked for regioselective sulfation.

3.1.2. Hexynylbenzoate Donors for α-Selective Glycosylation

Commonly, the construction of D-GlcN-α-1,4-D-GlcA/L-IdoA linkages involves the use of 2-azido-2-deoxy-glucopyranoside donors with thioglycoside or imidate as the preferred leaving group. In 2015 Yu and co-workers' reported using *ortho*-hexynylbenzoate donors of type **41** for the successful synthesis of D-GlcN-α-1,4-D-GlcA/L-IdoA linkages under Au(I) catalysis



Scheme 8. Synthesis of universal tetrasaccharide building block **40**. Reagents and conditions: (a) NaOMe, MeOH; (b) LevOH, EDC, DMAP, DCM, 91% 2 steps; (c) *p*-TsOH, MeOH; (d) TBDPSCl, imidazole, DCM, 86% 2 steps; (e) BzCl, pyridine, 95%; (f) $CH_2(CH_2SH)_2$, Et_3N, Pyridine, H_2O; (g) TFAA, pyridine, 81% 2 steps; (h) $HOCH_2CO_2{}^tBu$, NIS, AgOTf, DCM, 82%; (i) NaOMe, MeOH, DCM, 89%; (j) FmocCl, pyridine, 90%; (k) TFA, Et_3SiH, DCM, 72%; (l) NIS, AgOTf, DCM, RT, 60%.

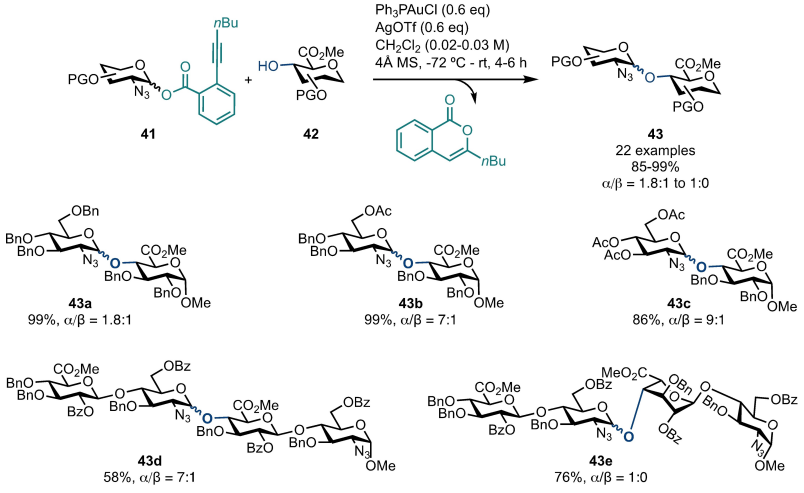
(Scheme 9).[24] The optimised reaction conditions used Ph_3PAuCl/AgOTf and a donor/acceptor ratio of 3 : 1. Given the known low reactivity of uronate D-GlcA/L-IdoA acceptors, high levels (3.0 equiv.) of donor were used to deliver high yields. A range of armed/disarmed donors and acceptors were screened (22 examples). Overall, the reaction yields were high (85–99%), but donor/acceptor reactivity and donor *O*-6 protecting group identity had a significant effect on the stereoselectivity. Reactive donors/acceptors gave poorer α-selectivity (**43 a** *versus.* **43 c**) and an ester at *O*-6 improved α-selectivity, suggested to be due to long range participation of such groups (**43 a** *versus.* **43 b**). [2 + 2] glycosylations to access tetrasaccharides showed an L-IdoA acceptor outperform its D-GlcA counterpart in terms of yield and α-selectivity (**43 d** *versus* **43 e**).

3.1.3. Programming for Regiodefined HS O/N *Sulfation*

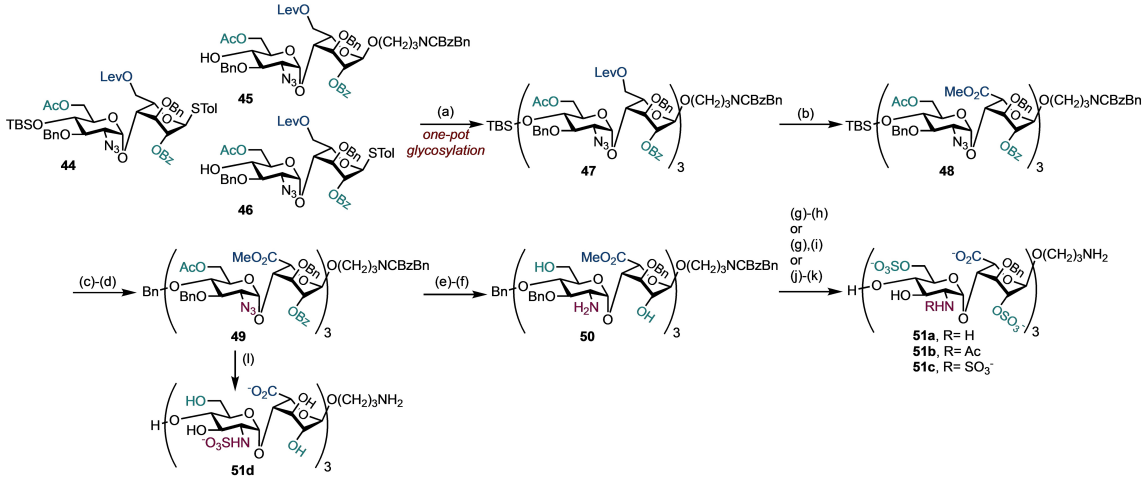
In 2015, Huang and co-workers' assembled seven hexasaccharides with different sulfation patterns to demonstrate sequence diversity. These materials originated from a single hexasaccharide precursor **47** (Scheme 10).[25]

To synthesise hexasaccharide precursor **47**, disaccharide thioglycoside donor **44** and disaccharide acceptors **45** and **46** were glycosylated in one-pot using *p*-TolSCl and AgOTf as promotors, delivering **47** in 67% yield. Following Lev-deprotection at C6 of idose, **47** was oxidised to uronate ester **48**. Next, the authors found it necessary to replace the non-reducing end O4-TBDMS group with Bn, prior to sulfation, to deliver **49**. Hexosamine nitrogen was unmasked, following azide reduction with 1,3-propanedithiol and the remaining deprotections and *O*/*N*-sulfations were completed chemically and enzymatically (chemical steps shown in Scheme 10) to provide a panel of hexasaccharides **51 a–d**.

Gardiner and co-workers' also reported a synthetic approach to a small matrix of protected heparin-type oligosaccharides containing orthogonal D-GlcN *O*-6 protecting groups.[26] Building on their earlier work,[27–30] this was completed to demonstrate capability in accessing programmability at specific sites, relevant to sulfation or other



Scheme 9. Yu and co-workers' Au catalysed synthesis of α-D-GlcN-(1→4)-D-GlcA/L-IdoA glycosidic linkages.



Scheme 10. Preactivation-based one-pot synthesis of hexasaccharide **47** to access differentially sulfated HS sequences. Reagents and conditions: (a) *p*-TolSCl, AgOTf, −78 °C, then **45** or **46**, TTBP, −30 °C, 1 h per glycosylation, 67 %; (b) $NH_2NH_2 \cdot AcOH$, pyridine, 0 °C, then BAIB, TEMPO, CH_2Cl_2, H_2O (2 : 1), rt, then MeI, K_2CO_3, DMF, rt, 70 %, 3 steps; (c) HF·pyridine, pyridine, 94 %; (d) BnBr, Ag_2O, 50 %; (e) MeONa; (f) 1,3-propanedithiol, Et_3N, 76 % 2 steps; (g) SO_3·pyridine, pyridine, 55 °C; (h) H_2, $Pd(OH)_2$, then LiOH, H_2O_2, **51a**: 72 %, 3 steps; (i) (a) Ac_2O, TEA; (b) H_2, $Pd(OH)_2$; (c) LiOH, H_2O_2, **51b**: 54 %, 4 steps; (j) SO_3·pyridine, pyridine, 55 °C; (k) H_2, $Pd(OH)_2$, then LiOH, H_2O_2, **51c**: 63 %, 3 steps; (l) (a) LiOH, H_2O_2, then KOH; (b) PMe_3, NaOH; (c) $SO_3 \cdot NEt_3$, NaOH, CH_3OH, rt; (d) H_2, $Pd(OH)_2$, 65 %, 4 steps.

modifications. Capability was demonstrated through the choice of D-GlcN 6-OH protecting group used; OBn or OAc programmed the fate of D-GlcN *O*-6 (OBn to deliver 6-OH and OAc for 6-OS).

In 2018, Boons and co-workers' reported an enzymatic modification of three chemically synthesised hexasaccharides, harnessing a D-GlcN-6-OMe blocking group, to effect regiodefined enzymatic modification and provide a library of 21 hexasaccharides.[31]

3.1.4. An S*-Linked HS Disaccharide*

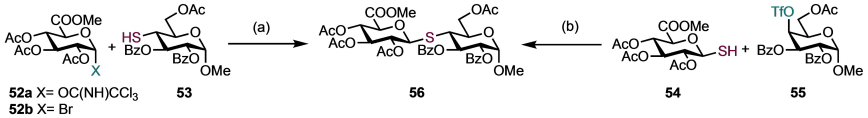
In comparison to *O*-glycosides, *S*-glycosides can show improved stability towards hydrolytic enzymes.[32] Kovensky and co-workers' reported the synthesis of an *S*-glycoside analogue of the disaccharide unit of HS and prepared multivalent glycoclusters based around this unit (Scheme 11).[33] The authors opted to use D-Glc instead of D-GlcN, as previous studies showed that such a substitution made only a small difference to biological activity.[34] Initial attempts to synthesise **56** using donors **52a–b** and acceptor **53** gave only trace amounts of the desired product. However, switching the approach to synthesise **56** through nucleophilic substitution of 4-*O*-triflated galactoside **55** with glycosyl thiol **54** afforded the desired *S*-glycoside in 79 % yield (α/β, 12 : 82).

3.2. Synthesis of Di- and Oligosaccharide HS Sequences

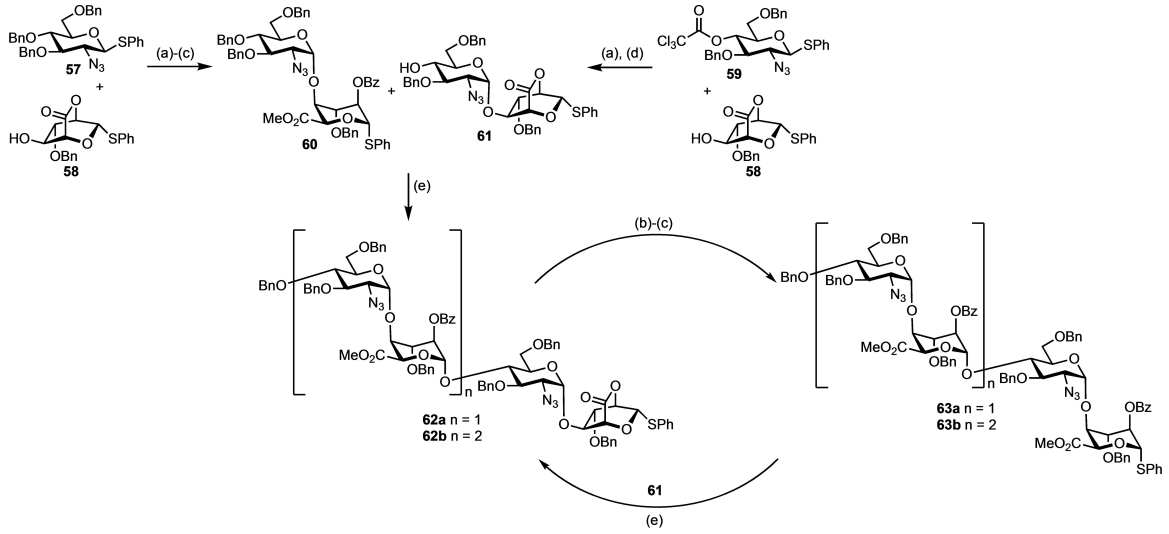
3.2.1. Iterative, Reducing End HS Oligosaccharide Synthesis

Gardiner and co-workers' described a novel approach towards the preparation of protected HS oligosaccharides bearing reducing end thioglycoside functionality (Scheme 12).[35] The authors employed a sequential chemoselective glycosylation at the reducing end, additive to their previously reported non-reducing terminus extension *via* lactone-terminated D-GlcN-L-IdoA intermediates.[36] This new approach introduced chemoselective activation of a reducing end L-IdoA-lactone to enable iterative HS oligosaccharide synthesis, without the need to interconvert the reducing end anomeric group.

Glycosylation of lactone thioglycoside acceptor **58** with GlcN donor **57** gave an intermediate disaccharide lactone which, upon subsequent opening and protection, afforded



Scheme 11. Synthesis of *S*-glycosidic analogue of disaccharide unit of HS. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −60 to 0 °C or Ag_2CO_3, AgOTf or HgO, $HgBr_2$; (b) Et_3N, DMF, DTT, −60 °C, 20 h, 79 %.



Scheme 12. Reagents and conditions: (a) NIS, AgOTf, toluene, **60** precursor: 74%, **61** precursor: 87%; (b) NaOMe, MeOH, CH_2Cl_2; (c) BzCl, 1-methylimidazole, DMAP, 1,2-DCE, **60**: 90% 2 steps, **63a**: 97% 2 steps, **63b**: 91%, 2 steps; (d) Pyridine, MeOH, **61**: 77%; (e) NIS, AgOTf, CH_2Cl_2, **62a**: 85%, 62b: 86%.

thioglycoside donor **60** in 67% yield over 3 steps. Acceptor **61** was prepared by glycosylation of 4-*O*-trichloroacetyl-protected glucoazide donor **59** and **58** to provide an intermediate disaccharide. From this the 4-*O*-trichloroacetyl group was removed to afford acceptor **61** in a 77% yield. Oligosaccharide assembly began with glycosylation of acceptor **61** using donor **60** to afford tetrasaccharide **62a** in 85% yield. Subsequent methanolysis and benzoylation of **62a** afforded tetrasaccharide **63a** in near quantitative yield. This material then underwent iteration from the reducing terminus using acceptor **61** to produce hexasaccharide **62b** in 86% yield. A final lactone opening and protection step afforded **63b** in 91% yield (Scheme 12).

3.2.2. Synthesis of Heparin-Related [4]$_n$ Oligosaccharides up to 40-Mer

In 2015 Gardiner and colleagues described the chemical synthesis of the longest heparin-related oligosaccharide to date (20-mer) using iterative synthesis and accessed protected oligosaccharides ranging from 16-mer through to the 40-mer.[37] Tetrasaccharide donor **64** was utitised in a coupling–deprotection cycle of 2-step homologation reactions, yielding oligosaccharides of increasing [4]$_n$ chain length (Scheme 13). The synthesis initiated from O4-protected 12-mer **65**[38] and, following selective deprotection at O4, generated 12-mer acceptor **66** in excellent yield. The subsequent homologation to 16-mer and thence to 20-mer proceeded in very good overall yield (68% and 79% respectively) for each 2-step coupling/deprotection sequence. Glycosylations of increasingly long acceptors with donor **64** proved reliable throughout a series of [4]$_n$ iterations and afforded 24-mer, 28-mer, 32-mer, 36-mer and 40-mer materials. Oligosaccharide **69a** was deprotected and *O*-/*N*-sulfated to afford heparin-like 20-mer **70**.

3.2.3. Exploring Functional Capability of Non-Reducing End D-GlcN Residues within HS Sequences

Gardiner's group also described installation of a non-reducing O4 handle in order to introduce conjugation-suitable, terminal functionality to biologically important HS oligosaccharides (Figure 2).[39] Their approach introduced a D-GlcN O4-aldehyde-level bearing unit (using an appropriate disaccharide donor) to remain latent throughout oligosaccharide synthesis. This latent aldehyde tag (LAT) was set to be unmasked at the final stage of synthesis, yielding fully sulfated, heparin-type

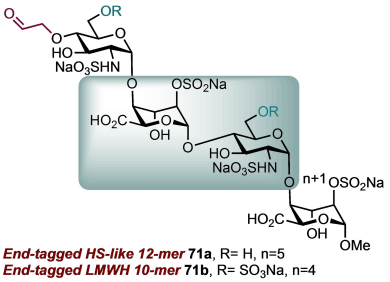
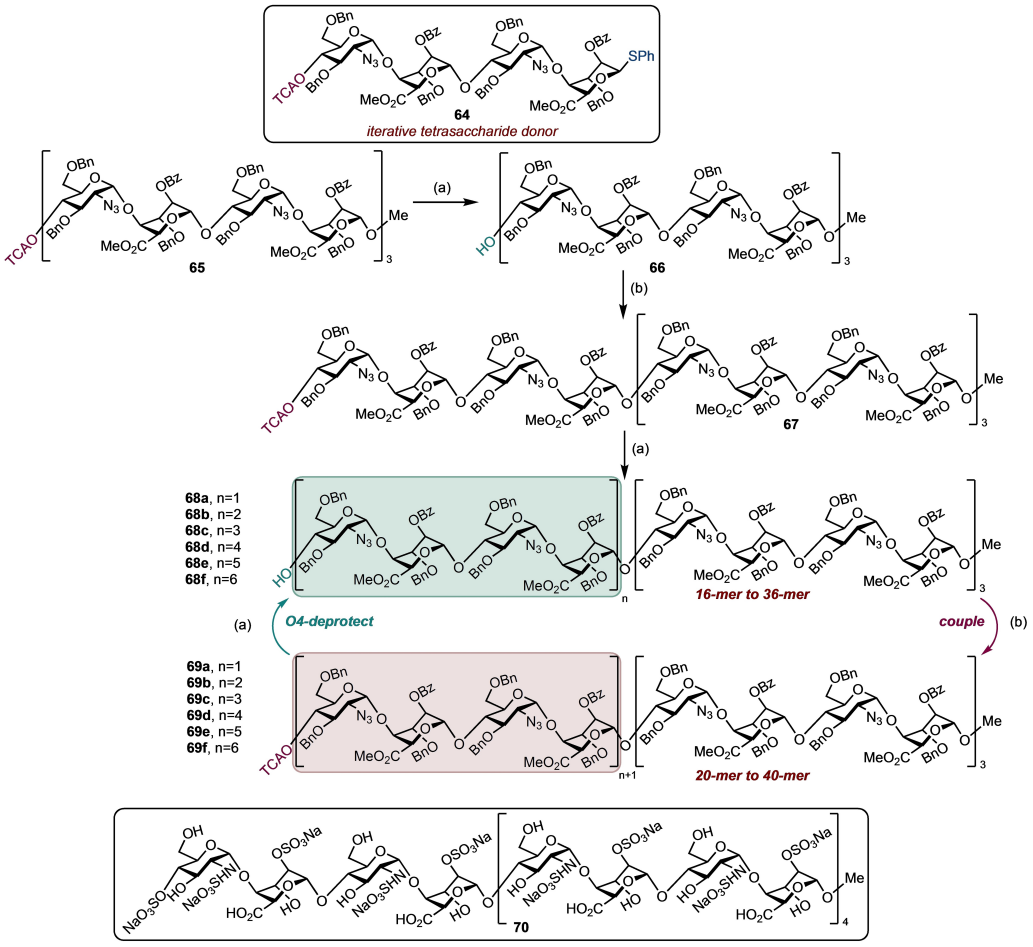


Figure 2. Gardiner's non-reducing end LAT technology, providing conjugation capability for HS oligosaccharides.



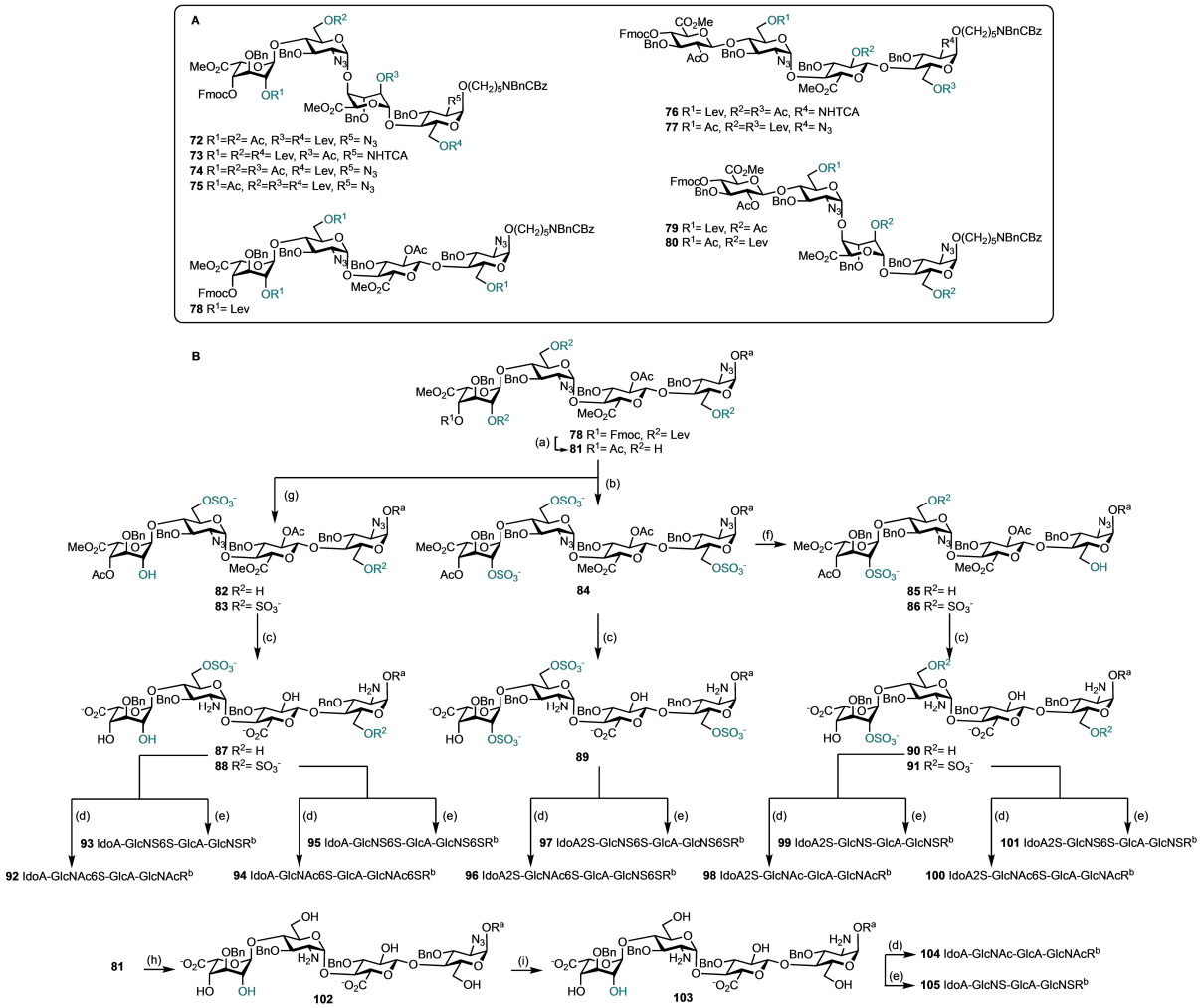
Scheme 13. Gardiner's HS 20-mer and protected 40-mer synthesis: Reagents and conditions: (a) MeOH, pyridine, **66**: 93%, **68a**: 90%, **68b**: 97%, **68c**: 95%, **68d**: 93%, **68e**: 93%, **68f**: 87%; (b) NIS, AgOTf (cat.), DCM, **64**, 67: 76%, **69a**: 81%, **69b**: 77%, **69c**: 75%, **69d**: 78%, **69e**: 72%, **69f**: 64%.

oligosaccharides with non-reducing end functional capability. This compared to an earlier, reducing end LAT unit introduced by the group to enable radiolabelled HS dodecasaccharide synthesis.[38]

The effect of moderating reducing end D-GlcN sulfation pattern was also investigated by the group.[40] Access to scalable, iterative synthesis delivered a site specifically O6 sulfated dodecasaccharide, which was used in conjunction with an earlier reported lower sulfated variant,[27] to then interrogate the effects of both level and site specificity of defined sulfation towards HS-dependant cytokines *in vitro* and *in vivo* .[40]

3.2.4. Modular Synthesis of HS Tetrasaccharide Libraries

To broadly explore HS-protein binding, Boons and co-workers' developed a library of 47 HS oligosaccharides for microarray immobilisation and screening.[41] The design conceived a limited number of tetrasaccharides which could be regioselectively *O*- and *N*-sulfated to rapidly diversify the final library. A total of 9 tetrasaccharides having 4 different backbone compositions with varying protecting group patterns were synthesised (Scheme 14A). With these in hand, regioselective *O*-sulfations were completed, giving access to materials of type **82** and **83** (Scheme 14B). Here the 6-OH at the distal D-GlcN$_3$ residue was found to be more reactive towards sulfation than the proximal D-GlcN$_3$ residue (**82**, 48% *versus* **83**, 35%). Regioselective sulfate ester removal was also explored using *N*,*O*-bis(trimethylsilyl)acetamide (BTSA) in pyridine. This gave access to **85** and **86**, where the 6-*O*-sulfate at the proximal D-GlcN$_3$ residue was more susceptible to hydrolysis than the distal position (**85**, 50% *versus* **86**, 27%). Similar diversification from tetrasaccharides **75**, **77** and **80** (with alternate backbone compositions) afforded an additional 19 sulfated oligosaccharides. Conventional modifications of tetrasaccharides **72–74**, **76** and **79** gave an additional 5



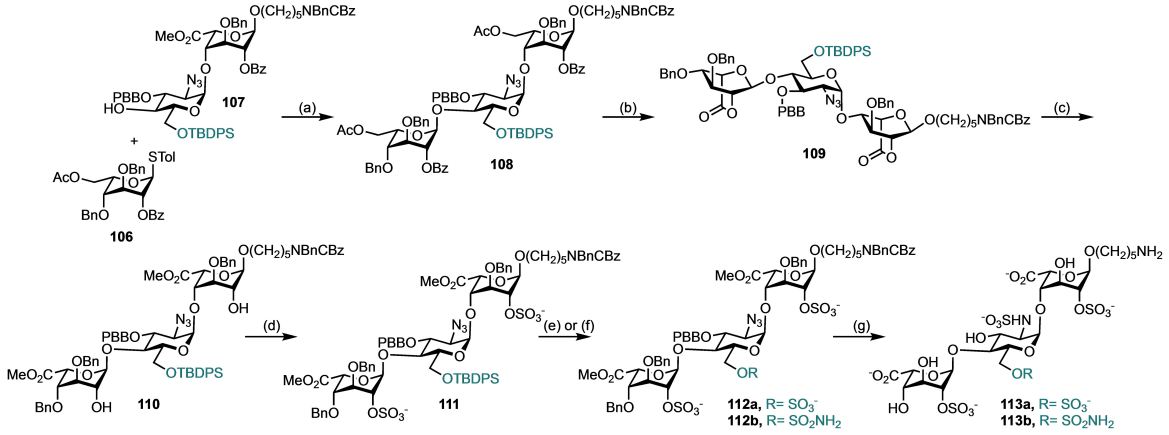
Scheme 14. A) Tetrasaccharide building blocks. B) Diversification example of tetrasaccharide **78** into 12 HS oligosaccharides. Reagents and conditions: (a) (i) Et_3N, MeOH, DCM; (ii) Ac_2O, pyridine, DMAP; (iii) $NH_2NH_2{\cdot}AcOH$, DCM, MeOH, rt, 2 h, 77 %, 3 steps; (b) $SO_3{\cdot}$pyridine, DMF, 2 h, 81 %; (c) (i) LiOH, H_2O_2, THF, 4 h, then NaOH, MeOH, 12 h; (ii) PMe_3, THF, MeOH, NaOH, 1 h, **87**: 78 %, **88**: 54 %, **89**: 70 %, **90**: 67 %, **91**: 54 %; (d) (i) Ac_2O, MeOH, Et_3N; (ii) Pd/C, H_2, MeOH, H_2O, 4 h; (iii) $Pd(OH)_2$, H_2, H_2O, 14 h, **92**: 65 %, **94**: 72 %, **96**: 85 %, **98**: 77 %, **100**: 72 %, **104**: 82 %; (e) (i) $SO_3{\cdot}$pyridine, MeOH, Et_3N, NaOH, 12 h; (ii) Pd/C, H_2, MeOH, H_2O, 4 h; (iii) $Pd(OH)_2$, H_2, H_2O, 14 h, **93**: 68 %, **95**: 77 %, **97**: 67 %, **99**: 69 %, **101**: 77 %, **105**: 71 %; (f) BTSA, pyridine, 60 °C, 2 h, **85**: 50 %; **86**: 27 %; (g) $SO_3{\cdot}$pyridine, DMF, **83**: 35 %, **87**: 48 %; (h) LiOH, H_2O_2, THF, 4 h, then NaOH, MeOH, 12 h, 84 %; (i) PMe_3, THF, MeOH, NaOH, 1 h, 87 %; R_a=$(CH_2)_5NBnCbz$; R_b=$(CH_2)_2NH_2$.

oligosaccharides that had lower levels of sulfation or possessed differently modified amino groups.

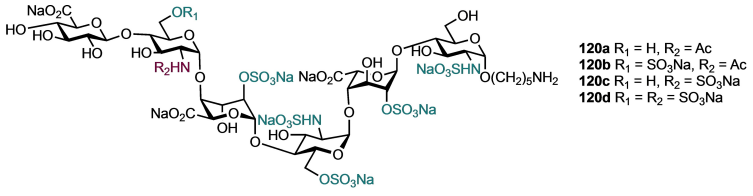
The group also recently reported an integrated methodology to broadly determine the ligand requirements of HS-binding proteins.[42] This involved firstly completing partial degradation of natural HS, followed by fragment screening against an immobilised HS-binding protein. Compositional analysis using HILIC-HRMS identified moderately sulfated octasaccharide sequences that informed subsequent chemical synthesis of a structurally defined ligand for further structure activity studies. This workflow was demonstrated successfully to establish the ligand requirements of the human Roundabout receptor 1 (Robo 1).

3.2.5. Access to a Sulfonamide-Containing HS Fragment

Hung and co-workers' examined the effectiveness of substrates and inhibitors of human endo-*O*-sulfatase-1 (Scheme 15).[43] The team synthesised a range of HS oligosaccharides with various chain lengths and *N*- and *O*- sulfation patterns and studied their substrate and inhibitor specificity using a competitive fluorogenic substrate, 4-methylumbelliferyl sulfate (4-MUS).



Scheme 15. Hung and co-workers' synthesis of **113a–b** to elucidate substrate specificity for Sulf-1. Reagents and conditions: (a) NIS, TfOH, CH_2Cl_2, −78 to −40 °C, 4 h, 82%; (b) (i) NaOMe, CH_2Cl_2, MeOH (1 : 1), rt, 18 h; (ii) TEMPO, BAIB, H_2O, CH_2Cl_2 (1 : 2), rt, 16 h, 70%, 2 steps; (c) MeOH, Et_3N, CH_2Cl_2, 40 °C, 18 h, 89%; (d) $SO_3 \cdot Et_3N$, DMF, 60 °C, 18 h, 79%; (e) (i) HF·pyridine, pyridine, THF, 72 h; (ii) $SO_3 \cdot Et_3N$, DMF, 60 °C, 18 h, **112a**: 73%, 2 steps; (f) (i) HF·pyridine, pyridine, THF, 72 h; (ii) $ClSO_2NHBn$, pyridine, rt, 10 min., **112b**: 83%, 2 steps; (g) (i) LiOH, H_2O_2, THF, 37 °C, 18 h; (ii) PMe_3, THF, THF, NaOH, rt, 14 h; (iii) $SO_3 \cdot$pyridine, Et_3N, NaOH, MeOH, rt, 18 h; (iv) $Pd(OH)_2/C$, H_2, MeOH, pH 7, rt, 3 d, **113a**: 38%, 4 steps, **113b**: 14%, 4 steps.



120a R_1 = H, R_2 = Ac
120b R_1 = SO_3Na, R_2 = Ac
120c R_1 = H, R_2 = SO_3Na
120d R_1 = R_2 = SO_3Na

Figure 3. Hexasaccharides **120a–d**.

To synthesise trisaccharides **113a** and **113b**, L-ido donor **106** and disaccharide acceptor **107** were glycosylated using NIS and TfOH to furnish α-linked trisaccharide **108**. Sequential deacylation using Zemplén's conditions and TEMPO oxidation converted **108** to lactone **109**. Ring opening of **109** delivered methyl ester **110** which was subjected to *O*-sulfation to yield a *bis*-*O*-sulfated derivative **111**. Desilylation and treatment with either $SO_3 \cdot Et_3N$ or $ClSO_2NHBn$, converted **111** to the sulfate **112a** or sulfonamide **112b**. Following final deprotections, target trisaccharides **113a** and **113b** were accessed in 11 and 5% overall yields respectively and in 11 steps.

3.3. *O*- and *N*-Sulfation Methods

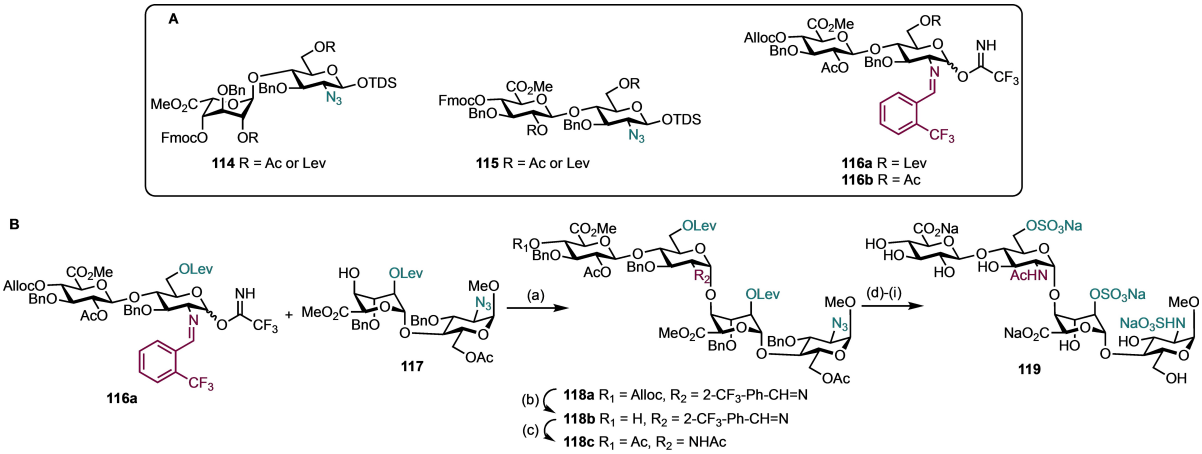
3.3.1. Synthetic HS Sequences Containing GlcNAc and GlcNS

In 2020 Boons and colleagues presented a modular approach to access HS oligosaccharides containing both D-GlcNAc and D-GlcNS units.[44] A previous modular synthetic route from the group provided a diverse range of HS oligosaccharides from two common disaccharide building blocks, **114** and **115** (Scheme 16A),[45] but did not deliver sequences containing both D-GlcNS and D-GlcNAc.

To address this (ultimately, to mimic high and low *N*-sulfation HS domains) the group synthesised a new pair of disaccharide building blocks **116a** and **116b** (Scheme 16A). Nguyen and co-workers' had established that α-glycosides were selectively formed using a Ni(II) triflate promoted activation of glycosyl donors containing the C2-trifluoromethylphenylmethanimine moiety present in **116a** and **116b**.[46] This, alongside the possibility for its orthogonal removal under mild acidic conditions, presented the prospect to build D-GlcN-azide and D-GlcN-imine containing oligosaccharides.

Accordingly, optimal conditions for the preparation of tetrasaccharide **118a** from donor **116a** and acceptor **117** (Scheme 16B) were established. This afforded the α-anomer of **118a** in an acceptable 58% yield. Scheme 16B also illustrates the subsequent conversion of **118a** into a final tetrasaccharide **119**, containing both D-GlcNS and D-GlcNAc components.

Finally, the team were able to assemble hexasaccharides **120a–d** (Figure 3) in good yields from modular disaccharide donors and conventional acceptors *via* a series of sequential



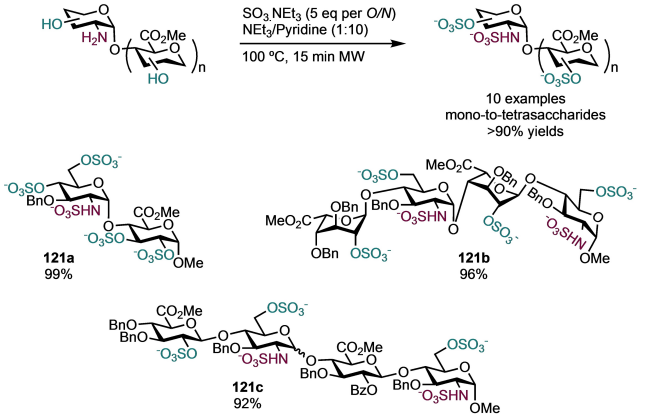
Scheme 16. A) Boon's modular disaccharide building blocks B) Glycosidation of trifluoro-*N*-phenylacetimidate disaccharides. Reagents and conditions: (a) TfOH, −20 °C, 12 h, 58 %; (b) $Pd(PPh_3)_4$, THF, H_2O, 2 h; (c) HCl, THF, H_2O, 15 min., then Ac_2O, pyridine, 3 h, 90 %, 2 steps; (d) $NH_2NH_2 \cdot AcOH$, toluene, EtOH, 2 h; (e) $SO_3 \cdot$pyridine, DMF, 2 h; (f) LiOH, H_2O_2, THF, H_2O, 60 %, 5 steps; (g) PMe_3, NaOH, THF, H_2O, 2 h; (h) $SO_3 \cdot$pyridine, MeOH, Et_3N, 66 %, 2 steps; (i) $Pd(OH)_2/C$, H_2, ^{t}BuOH, H_2O, 24 h, 90 %.

deprotection and sulfation steps. To study the effects of NS *versus* NAc substitution and sulfation pattern, compounds **120 a–d**, were printed as a glycan microarray onto an *N*-hydroxysuccinamide activated glass slide and their binding to chemokines IL-8 and RANTES were investigated. Studies revealed that IL-8 was able to bind a hexasaccharide in which D-GlcNS was replaced by D-GlcNAc units (**120 d** *versus* **120 b**); moreover absence of 6-OS caused a reduction in binding (**120 d** *versus* **120 c**).

3.3.2. Methodology Improvements to Effect O *and* N*-Sulfation*

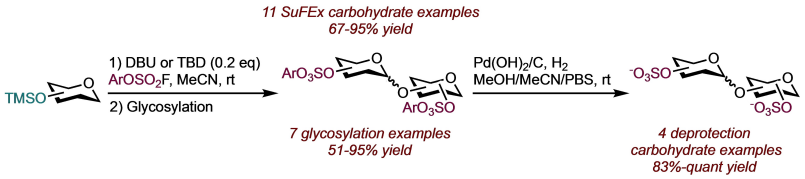
Yu and co-workers' reported an effective method to simultaneously complete *O*- and *N*-sulfation under microwave conditions (Scheme 17).[47] Using a cocktail of $SO_3 \cdot Et_3N$, NEt_3 and pyridine, a simultaneous *O*,*N*-sulfation could be completed in a very short time (15 minutes). In the absence of NEt_3 only *O*-sulfation took place; hence, the presence of NEt_3 was deemed crucial to obtain simultaneous heteroatom sulfation. Interestingly, using the same cocktail with conventional heating gave only *N*-sulfation. A rationale for these observations was that as *O*-sulfonation occured, the system became acidic, leading to protonation of the amino group which was then not free to react with sulfur trioxide. However, the presence of NEt_3 deprotonates the hexosamine nitrogen, thus enabling *N*-sulfation. The reaction conditions worked well on a range of mono-, di-, tri- and tetrasaccharides (> 90 % yields) and sterically hindered hydroxyl groups (**121 a**) were sulfated without issue.

In 2020, Niu and co-workers' reported an approach to early stage *O*-sulfation *via* the sulfur(VI) fluoride exchange



Scheme 17. Microwave assisted simultaneous *O*- and *N*-sulfation by Yu and co-workers'.[47]

(SuFEx) reaction between TMS-protected sugars and aryl fluorosulfates (Scheme 18).[48] Arylfluorosulfates bearing electron withdrawing substituents worked best for sulfate diester formation. The authors also demonstrated a one-pot, *in-situ* hydroxyl silylation with hexamethyldisilazane (HMDS) and subsequent SuFEx reaction. This one-pot protocol is useful as it circumvents any need to purify intermediate TMS silyl-ethers, which are known to be unstable on silica. The sulfate diesters showed excellent robustness to subsequent acidic, basic, oxidising and reducing reaction conditions. Various glycosyl donors and acceptors decorated with sulfate diesters were successfully employed for glycosylation reactions. Sulfate diester deprotection was achieved using late-stage hydrogenolysis. This early-stage *O*-sulfation method provides a



Scheme 18. Niu and co-workers' early-stage *O*-sulfation using SuFEx chemistry.

general, powerful tool for the synthesis of *O*-sulfated bioactive compounds.

4. Automated and Solid Phase Synthesis Approaches to HS Sequences

4.1 Solid Phase Synthesis of a H/HS Hexasaccharide Precursor

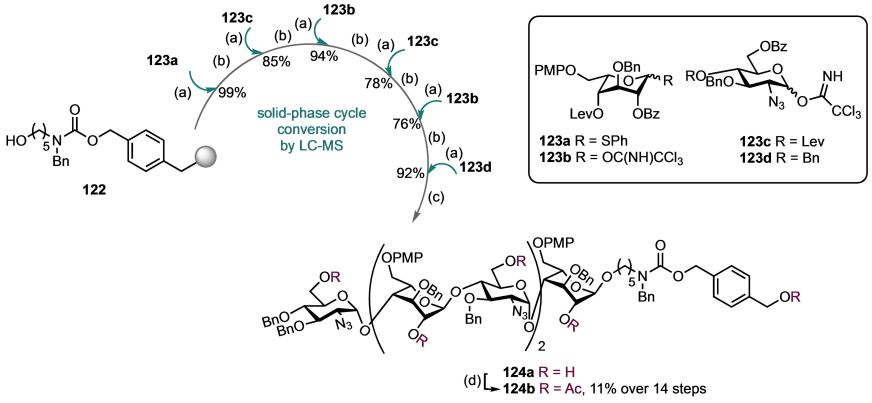
The solid-phase synthesis (SPS) of carbohydrate targets is renowned for its challenges in comparison to solution-phase approaches. Unlike automated peptide and oligonucleotide assembly, oligosaccharide synthesis requires the development of procedures to account for multiple hydroxyl groups of similar reactivity and the requirement for high yielding and stereoselective glycosylation reactions. In 2015, Reichardt and co-workers' developed an effective strategy in this area for the solid-phase assembly of an HS precursor using monosaccharide building blocks (Scheme 19).[49]

Previous work by the group established that monosaccharide **123 a** had good reactivity and stability for the solid-phase synthesis of a HS trisaccharide precursor.[50] Therefrom, the sequential assembly of a hexasaccharide was performed using monosaccharides **123 a–d**. Attachment of a pentyl spacer unit, protected with a 4-hydroxymethylbenzyl *N*-benzyl carbamate, to a carboxystyrene resin *via* an ester linkage provided a reliable solid-support (**122**). Successive capping, delevulination of idose-O4 and alternate glycosylation cycles with donors **123 a**, **123 b** and **123 c**, followed by final glycosylation with D-GlcN donor **123 d** furnished H/HS precursor **124 a**, after cleavage from the resin. Acetylation of this material and HPLC purification gave the desired hexasaccharide **124 b** in an 11 % yield over 14 steps (85 % average yield per step). This effective method delivered significant progress towards a routine and automatable solid-phase approach, notably completable within 2–3 weeks, as opposed to several months in solution phase.

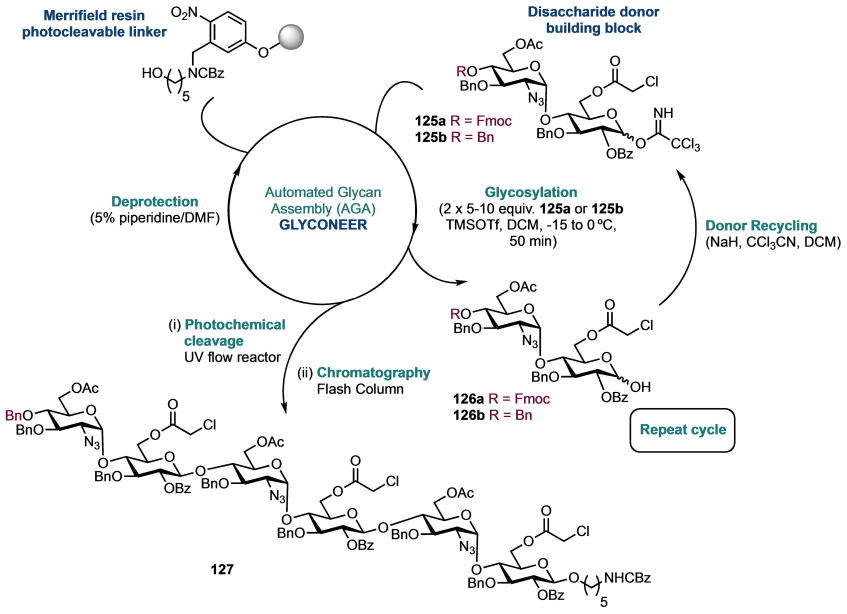
4.2 Automated Glycan Assembly of H/HS

An alternative approach towards an HS hexasaccharide precursor was reported by Fascione and Schwörer using automated glycan assembly (AGA) (Scheme 20).[51] AGA eliminates the reliance on manual methods (chemoenzymatic, solution-phase and solid-phase) and provides a rapid approach towards the formation of biologically important carbohydrates. The team reported iterative, automated solid-phase synthesis of an HS hexasaccharide precursor using Glyconeer™, the automated oligosaccharide synthesiser.

Disaccharide **125 a** was employed as the initial donor towards the synthesis of hexasaccharide **127**. A labile Fmoc protecting group was used as the temporary protecting group



Scheme 19. Solid-phase assembly of a H/HS hexasaccharide precursor by Reichardt and co-workers'. Reagents and conditions: (a) NIS, TMSOTf, −20 °C to rt; (b) $NH_2NH_2 \cdot AcOH$, DCM, MeOH (4 : 1); (c) NaOMe, MeOH; (d) Ac_2O, pyridine, 0 °C to rt.



Scheme 20. Fascione and Schwörer's AGA of an HS hexasaccharide precursor.

at *O*-4, to release acceptor capability for D-GlcN for chain elongation throughout the iterative glycosylations. Fmoc deprotection could be conveniently monitored by an inline UV detector, which measured the amount of dibenzofulvene adduct released in solution. A Merrifield resin was chosen as the solid-support and this was additionally functionalised with a photolabile nitrobenzyl ether-based linker (stable to acidic and basic reaction solutions). Excess hydrolysed donor **126a** from glycosylation steps was recycled back to **125a** in solution, at an efficiency of ~60%, highlighting the resourcefulness of AGA. Finally, cleavage from the resin was performed using an in house photoreactor, followed by filtration and evaporation to afford the crude hexasaccharide. Purification *via* silica gel flash column chromatography afforded **127** in 6 hours with a 30% yield on 15 mg scale.

Interestingly, this approach proposed to complete a late-stage oxidation of D-Glc to D-GlcA (after oligosaccharide assembly) and a recent report from Schwörer has indeed highlighted the need for consideration of the complimentarity of this approach *versus* using D-GlcA disaccharide donors directly.[52]

5. Conclusion and Outlook

Developments in the field of chemical H/HS synthesis over the past five years have largely focused around and achieved the provision of ever longer and more diverse libraries of sulfation variant ligands. This has been expedited by improvements in access to requisite building blocks, notably introducing an upcycling of HS-related polysaccharides to deliver such materials. The matrix of structural possibilities for HS is enormous and often daunting, but the advent of integrated platforms to guide ligand design (and therefore synthesis) will certainly simplify future endeavours here.

Technologies to synthesise these essential glycan fragments have also undergone improvements. From the perspective of medically relevant heparin sequences, iterative, programmable, one-pot solution-phase syntheses to access defined fragment lengths surrounding Fondaparinux have emerged. Coupled with the recent establishments of automated glycan assembly and traditional solid phase synthesis, it is likely that there will be easier and more rapid access to ever more diverse and challenging sequences to support biological requirements in the near future. This must of course be considered in tandem and synergise with achievements made in the chemoenzymatic preparation of H/HS.

An additional area that will surely continue to grow as a result of these accomplishments is around the fusion of HS synthesis with protein conjugation/synthesis, which will enable study of the larger and more challenging HS-proteoglycan axis. Such endeavours were recently exemplified through the synthesis and evaluation of a human syndecan-4 glycopeptide.[53,54]

Acknowledgements

The Engineering and Physical Sciences Research Council (EPSRC, EP/T007397/1) and UK Research and Innovation (UKRI, Future Leaders Fellowship, MR/T019522/1) are thanked for project grant funding to G. J. M. Keele University are thanked for PhD studentship funding to C. O. S. and H. W.

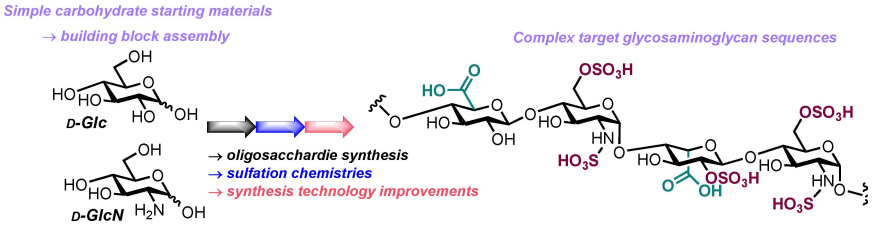
References

[1] J. T. Gallagher, J. E. Turnbull, *Glycobiology* **1992**, *2*, 523–528.
[2] N. Afratis, C. Gialeli, D. Nikitovic, T. Tsegenidis, E. Karousou, A. D. Theocharis, M. S. Pavão, G. N. Tzanakakis, N. K. Karamanos, *FEBS J.* **2012**, *279*, 1177–1197.
[3] R. Schwörer, O. V. Zubkova, J. E. Turnbull, P. C. Tyler, *Chem. Eur. J.* **2013**, *19*, 6817–6823.
[4] T. M. Clausen, D. R. Sandoval, C. B. Spliid, J. Pihl, H. R. Perrett, C. D. Painter, A. Narayanan, S. A. Majowicz, E. M. Kwong, R. N. McVicar, B. E. Thacker, C. A. Glass, Z. Yang, J. L. Torres, G. J. Golden, P. L. Bartels, R. N. Porell, A. F. Garretson, L. Laubach, J. Feldman, X. Yin, Y. Pu, B. M. Hauser, T. M. Caradonna, B. P. Kellman, C. Martino, P. L. S. M. Gordts, S. K. Chanda, A. G. Schmidt, K. Godula, S. L. Leibel, J. Jose, K. D. Corbett, A. B. Ward, A. F. Carlin, J. D. Esko, *Cell* **2020**, *183*, 1–15.
[5] C. J. Mycroft-West, D. Su, I. Pagani, T. R. Rudd, S. Elli, N. S. Gandhi, S. E. Guimond, G. J. Miller, M. C. Z. Meneghetti, H. B. Nader, Y. Li, Q. M. Nunes, P. Procter, N. Mancini, M. Clementi, A. Bisio, N. R. Forsyth, V. Ferro, J. E. Turnbull, M. Guerrini, D. G. Fernig, E. Vicenzi, E. A. Yates, M. A. Lima, M. A. Skidmore, *Thromb. Haemostasis* **2020**, *120*, 1700–1715.
[6] F. Baleux, L. Loureiro-Morais, Y. Hersant, P. Clayette, F. Arenzana-Seisdedos, D. Bonnaffé, H. Lortat-Jacob, *Nat. Chem. Biol.* **2009**, *5*, 743–748.
[7] M. T. Shieh, D. WuDunn, R. I. Montgomery, J. D. Esko, P. G. Spear, *J. Cell Biol.* **1992**, *116*, 1273–1281.
[8] B. García, J. Merayo-Lloves, C. Martin, I. Alcalde, L. M. Quirós, F. Vazquez, *Front. Microbiol.* **2016**, *7*, 220.
[9] E. P. Chappell, J. Liu, *Bioorg. Med. Chem.* **2013**, *21*, 4786–4792.
[10] Z. Wang, K. Arnold, V. M. Dhurandhare, Y. Xu, J. Liu, *RSC Chem. Biol.* **2021**, *2*, 702–712.
[11] M. Mende, C. Bednarek, M. Wawryszyn, P. Sauter, M. B. Biskup, U. Schepers, S. Bräse, *Chem. Rev.* **2016**, *116*, 8193–8255.
[12] J. Choay, J.-C. Jacquinet, J.-C. Lormeau, M. Nassr, M. Petitou, P. Sinay, *US Pat. US4818816 A*, **1989**.
[13] X. Dai, W. Liu, Q. Zhou, C. Cheng, C. Yang, S. Wang, M. Zhang, P. Tang, H. Song, D. Zhang, Y. Qin, *J. Org. Chem.* **2016**, *81*, 162–184.
[14] Y. Ding, C. V. N. S. Vara Prasad, H. Bai, B. Wang, *Bioorg. Med. Chem. Lett.* **2017**, *27*, 2424–2427.
[15] S. Dey, C. H. Wong, *Chem. Sci.* **2018**, *9*, 6685–6691.
[16] C. M. Pedersen, L. U. Nordstrøm, M. Bols, *J. Am. Chem. Soc.* **2007**, *129*, 9222–9235.
[17] L. Wang, Y. Hashidoko, M. Hashimoto, *J. Org. Chem.* **2016**, *81*, 4464–4474.
[18] H. Jin, Q. Chen, Y. Y. Zhang, K. F. Hao, G. Q. Zhang, W. Zhao, *Org. Chem. Front.* **2019**, *6*, 3116–3120.
[19] S. Dey, H. J. Lo, C. H. Wong, *Org. Lett.* **2020**, *22*, 4638–4642.
[20] S. Dey, H. J. Lo, C. H. Wong, *J. Am. Chem. Soc.* **2019**, *141*, 10309–10314.
[21] C. D. Shanthamurthy, R. Kikkeri, *Eur. J. Org. Chem.* **2019**, *2019*, 2950–2953.
[22] R. C. Sawant, Y. J. Liao, Y. J. Lin, S. S. Badsara, S. Y. Luo, *RSC Adv.* **2015**, *5*, 19027–19033.
[23] N. J. Pawar, L. Wang, T. Higo, C. Bhattacharya, P. K. Kancharla, F. Zhang, K. Baryal, C. X. Huo, J. Liu, R. J. Linhardt, X. Huang, L. C. Hsieh-Wilson, *Angew. Chem. Int. Ed.* **2019**, *58*, 18577–18583; *Angew. Chem.* **2019**, *131*, 18750–18756.
[24] J. Li, Y. Dai, W. Li, S. Laval, P. Xu, B. Yu, *Asian J. Org. Chem.* **2015**, *4*, 756–762.
[25] S. B. Dulaney, Y. Xu, P. Wang, G. Tiruchinapally, Z. Wang, J. Kathawa, M. H. El-Dakdouki, B. Yang, J. Liu, X. Huang, *J. Org. Chem.* **2015**, *80*, 12265–12279.
[26] M. Baráth, S. U. Hansen, C. E. Dalton, G. C. Jayson, G. J. Miller, J. M. Gardiner, *Molecules* **2015**, *20*, 6167–6180.
[27] S. U. Hansen, G. J. Miller, G. C. Jayson, J. M. Gardiner, *Org. Lett.* **2013**, *15*, 88–91.
[28] S. U. Hansen, G. J. Miller, M. Baráth, K. R. Broberg, E. Avizienyte, M. Helliwell, J. Raftery, G. C. Jayson, J. M. Gardiner, *J. Org. Chem.* **2012**, *77*, 7823–7843.
[29] G. J. Miller, S. U. Hansen, E. Avizienyte, G. Rushton, C. Cole, G. C. Jayson, J. M. Gardiner, *Chem. Sci.* **2013**, *4*, 3218–3222.
[30] G. J. Miller, S. U. Hansen, M. Baráth, C. Johannessen, E. W. Blanch, G. C. Jayson, J. M. Gardiner, *Carbohydr. Res.* **2014**, *400*, 44–53.
[31] W. Lu, C. Zong, P. Chopra, L. E. Pepi, Y. Xu, I. J. Amster, J. Liu, G. J. Boons, *Angew. Chem. Int. Ed.* **2018**, *57*, 5340–5344; *Angew. Chem.* **2018**, *130*, 5438–5442.
[32] H. Driguez, in *Glycosci. Synth. Substrate Analog. Mimetics* (Eds.: H. Driguez, J. Thiem), Springer Berlin Heidelberg, Berlin, Heidelberg, **1998**, pp. 85–116.
[33] D. S. E. K. Teki, A. Bil, V. Moreau, V. Chagnault, B. Fanté, A. Adjou, J. Kovensky, *Org. Chem. Front.* **2019**, *6*, 2718–2725.
[34] M. Petitou, C. A. A. Van Boeckel, *Angew. Chem. Int. Ed.* **2004**, *43*, 3118–3133; *Angew. Chem.* **2004**, *116*, 3180–3196.
[35] R. A. Jeanneret, C. E. Dalton, J. M. Gardiner, *J. Org. Chem.* **2019**, *84*, 15063–15078.
[36] S. U. Hansen, C. E. Dalton, M. Baráth, G. Kwan, J. Raftery, G. C. Jayson, G. J. Miller, J. M. Gardiner, *J. Org. Chem.* **2015**, *80*, 3777–3789.
[37] S. U. Hansen, G. J. Miller, M. J. Cliff, G. C. Jayson, J. M. Gardiner, *Chem. Sci.* **2015**, *6*, 6158–6164.
[38] S. U. Hansen, G. J. Miller, C. Cole, G. Rushton, E. Avizienyte, G. C. Jayson, J. M. Gardiner, *Nat. Commun.* **2013**, *4*, 1–9.

[39] G. J. Miller, K. R. Broberg, C. Rudd, M. R. Helliwell, G. C. Jayson, J. M. Gardiner, *Org. Biomol. Chem.* **2015**, *13*, 11208–11219.
[40] G. C. Jayson, S. U. Hansen, G. J. Miller, C. L. Cole, G. Rushton, E. Avizienyte, J. M. Gardiner, *Chem. Commun.* **2015**, *51*, 13846–13849.
[41] C. Zong, A. Venot, X. Li, W. Lu, W. Xiao, J. S. L. Wilkes, C. L. Salanga, T. M. Handel, L. Wang, M. A. Wolfert, G. J. Boons, *J. Am. Chem. Soc.* **2017**, *139*, 9534–9543.
[42] C. Zong, R. Huang, E. Condac, Y. Chiu, W. Xiao, X. Li, W. Lu, M. Ishihara, S. Wang, A. Ramiah, M. Stickney, P. Azadi, I. J. Amster, K. W. Moremen, L. Wang, J. S. Sharp, G. J. Boons, *J. Am. Chem. Soc.* **2016**, *138*, 13059–13067.
[43] L. T. Chiu, N. M. Sabbavarapu, W. C. Lin, C. Y. Fan, C. C. Wu, T. J. R. Cheng, C. H. Wong, S. C. Hung, *J. Am. Chem. Soc.* **2020**, *142*, 5282–5292.
[44] L. Sun, P. Chopra, G. J. Boons, *J. Org. Chem.* **2020**, *85*, 16082–16098.
[45] S. Arungundram, K. Al-Mafraji, J. Asong, F. E. Leach, I. J. Amster, A. Venot, J. E. Turnbull, G. J. Boons, *J. Am. Chem. Soc.* **2009**, *131*, 17394–17405.
[46] E. T. Sletten, S. K. Ramadugu, H. M. Nguyen, *Carbohydr. Res.* **2016**, *435*, 195–207.
[47] P. Xu, S. Laval, Z. Guo, B. Yu, *Org. Chem. Front.* **2016**, *3*, 103–109.
[48] C. Liu, C. Yang, S. Hwang, S. L. Ferraro, J. P. Flynn, J. Niu, *Angew. Chem. Int. Ed.* **2020**, *59*, 18435–18441; *Angew. Chem.* **2020**, *132*, 18593–18599.
[49] N. Guedes, S. Kopitzki, B. Echeverria, R. Pazos, E. Elosegui, J. Calvo, N. C. Reichardt, *RSC Adv.* **2015**, *5*, 9325–9327.
[50] N. Guedes, P. Czechura, B. Echeverria, A. Ruiz, O. Michelena, M. Martin-Lomas, N. C. Reichardt, *J. Org. Chem.* **2013**, *78*, 6911–6934.
[51] D. Budhadev, K. Saxby, J. Walton, G. Davies, P. C. Tyler, R. Schwörer, M. A. Fascione, *Org. Biomol. Chem.* **2019**, *17*, 1817–1821.
[52] D. J. Sheppard, S. A. Cameron, P. C. Tyler, R. Schwörer, *Org. Biomol. Chem.* **2020**, *18*, 4728–4733.
[53] W. Yang, K. Yoshida, B. Yang, X. Huang, *Carbohydr. Res.* **2016**, *435*, 180–194.
[54] W. Yang, Y. Eken, J. Zhang, L. E. Cole, S. Ramadan, Y. Xu, Z. Zhang, J. Liu, A. K. Wilson, X. Huang, *Chem. Sci.* **2020**, *11*, 6393–6404.

Manuscript received: June 24, 2021
Revised manuscript received: August 19, 2021
Version of record online: ■■ ■■, ■■■■

RECORD REVIEW



This review describes recent advances (2015 onwards) surrounding the chemical synthesis of heparin and heparan sulfate.

*Dr. I. Pongener, C. O'Shea, H. Wootton, Prof. M. Watkinson, Prof. G. J. Miller**